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A Micro Glass Electrode

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(Received 3 May 1952)

Glass electrode assemblies as normally supplied by manufacturers require that the glass electrode, as a small bulb, together with either the standard calomel electrode or a potassium chloride-agar bridge, shall dip into the liquid under examination. Under these conditions a reduction in the volume of liquid below 5-10 ml. means that the containing vessel becomes so small that the risk of scratching the thin glass bulb becomes considerable. Rather smaller volumes can safely be used in the Morton (1930) electrode since only the glass electrode dips into the liquid; contact with the calomel electrode being through a stopcock at the base of the electrode vessel.

Most adaptations of the glass electrode to very small volumes have involved a reduction in the area of the glass membrane. Thus Paic (1938) fused a thin septum of glass at a short distance from one end of a narrow U-tube so forming a very small cup in which 0.1 ml. fluid was sufficient to cover the membrane. Chinoy (1947) made use of an eccentrically blown bulb with a depression at its uppermost point in which 0.05 ml. was placed. In both cases a bridge was used to make contact with the calomel electrode. Such electrodes naturally have a much greater electrical resistance than a fully immersed standard type of bulb, and there is again risk of damaging the membrane with the bridge when the depth of fluid is very small.

Other workers, for example Dietz (1948), have used a block of insulating material in which are two

depressions connected by a small channel. The depressions are designed to fit the two electrodes so that each is surrounded by a film of liquid. The smaller the volume of liquid available for such a set up the greater are the chances of damage to the glass bulb as it is lowered into its cavity. By fitting a conventional bulb type electrode concentrically within a wider tube to allow about 1 mm. clearance Hanes (1951) could measure the pH of 1 drop of fluid which was retained in the annular space by capillarity. Such an electrode would be safe from damage as long as it remained rigidly fixed within the outer tube. Britton (1942) has reviewed various types of glass electrode.

Michaelis (1936) designed an electrode in which the glass membrane took the form of a thin-walled tube fused within a wider vertical tube. Such a design ensures complete protection of the glass membrane from mechanical damage. The centre tube held 3-5 ml. of the liquid under examination, while the annular space contained dilute hydrochloric acid into which dipped the silver-silver chloride electrode. At the lower end of the tube was fitted a 5-way stopcock to allow connexion via a potassium chloride bridge to the calomel electrode. Pickford (1937) has described a modification of Michaelis's design, for much smaller volumes (approx. 0.1 ml.). The present design is based upon that of Michaelis but is simpler than the two just mentioned and can be used with 0.3 ml. liquid. Measurements can be made very rapidly

and, if necessary, the sample can be recovered quantitatively. Furthermore, the making up of the electrode requires no great skill in glass-blowing.

Details of construction

All glass tubing was cleaned before use in sulphuric-chromic acid mixture followed by 6 N-HCl.

Since difficulty was experienced in obtaining a sound internal seal between soda glass and the inner tube of high conductivity electrode glass, short lengths of soda glass were interposed between the seals and the capillary of electrode glass.

A 2 cm. length of Corning 015 glass tubing* (8 mm. external diameter, 0.7 mm. wall) was sealed at each end to longer pieces of soda glass of the same diameter. With a glass-cutting knife a ring was scratched round each of the soda glass pieces about 8 mm. from the union with the electrode glass. Using a very narrow flame (e.g. fishtail burner) the centre of the piece of electrode glass was heated and pulled out into a thin-walled capillary about 20 cm. long and 0.5–1.0 mm. diameter. The soda glass was then cut off at the scratch marks and the ends of the tube opened out slightly (Fig. 1). If the capillary is narrower it becomes very fragile and the rate of draining would in any case be inconveniently slow.

The outer tube of the assembly was a length of soda glass about 15 mm. diameter with a curved side-arm of 6–7 mm. internal diameter. The capillary was sealed into this tube as shown in Fig. 2 and a stopcock attached to the lower end. The capillary and annular space were filled with 0.1 N-HCl and left standing 24 hr.

By measuring the resistance of the electrode membrane it is possible to reject at this stage electrodes which are obviously unsuitable, either because the membrane is too thick or because it is not continuous and the resistance therefore very low. Electrodes with resistances of 20–30 MΩ. give satisfactory results when used with a Cambridge pH meter or similar instrument. The resistance of an electrode was conveniently measured by connecting it in series with a fixed resistor of 50 MΩ., a sensitive mirror galvanometer and a 2 V. battery. Connexions with the acid on each side of the membrane were made with platinum wires. If the galvanometer deflexions obtained before and after short-circuiting the electrode are d and D respectively the resistance of the electrode is $50 (D - d)/d$ MΩ.

The side arm on the apparatus serves to hold the Ag-AgCl electrode, and for connexion of this electrode to the pH meter the co-axial cable (5.5 mm. diameter) used for television aerial leads is suitable. The outer insulation and the copper screen immediately beneath it were stripped back about 3 cm. at one end of a suitable length of cable to expose the white polythene layer. The latter was then stripped to expose about 1.5 cm. of the central copper wire to which was attached a spade terminal (or other suitable connector for the negative electrode terminal on the meter). Since the central wire is thin it is advisable to wind it back over the polythene insulation (Fig. 3e) and then grip it in the spade terminal. To the outer screen was soldered a piece of copper wire of a length sufficient to reach the 'earth' terminal on the meter when the spade terminal is connected.

* Other types of electrode glass would no doubt prove satisfactory.

To prevent flexing of this wire and possible breakage a short length of polythene sleeving was slipped over the wire and by gentle heating this was moulded to the polythene sleeving in the cable (Fig. 3f). The central wire was similarly bared at the other end of the cable (Fig. 2c) and soldered to a 5 cm. length of silver wire (Fig. 2b). When tested with a 'Megger' the resistance between the central wire and the screen should be infinity. The silver-copper joint was covered with sealing wax and the Ag-AgCl electrode prepared by electrolysis N-HCl with the silver wire as anode. Current was passed for 3–5 min. from a 2 V. battery.

Fig. 1.

Fig. 3.

Fig. 2.

Fig. 1. Details of one end of electrode capillary.

Fig. 2. The completed glass electrode. (a) Capillary to hold liquid under test. (b) Ag-AgCl electrode dipping into 0.1 N-HCl. (c) Co-axial cable, the screening conductor of which is earthed. The cable is held in place with sealing wax. (d) Tip of KCl-agar bridge to calomel electrode.

Fig. 3. End of co-axial cable connecting with pH meter. (e) Central conductor wound back over polythene insulation preparatory to fixing spade terminal. (f) Wire connecting the screen, which surrounds the polythene insulation, to the 'earth' terminal on the pH meter.

The glass electrode was emptied, and the outer tube re-filled with 0.1 N-HCl so that the acid came to within 3–4 cm. of the top of the side arm. The Ag-AgCl electrode was supported in a clamp so that it made contact with the HCl in the electrode and a test was carried out as described below. If it was satisfactory the Ag-AgCl electrode was fixed into the side arm with sealing wax so that only the silver wire was exposed beneath the seal. In a second test the 'Megger' should again read infinity.

Calomel electrode for use with the glass electrode

A convenient type of calomel electrode assembly consisted of a short test tube (10 × 2.5 cm. diameter) carrying in a two-hole rubber bung a calomel electrode (Cambridge Instrument Company) and an inverted U-tube filled with agar-KCl. The test tube contained saturated KCl plus excess of solid KCl and the end of the U-tube outside the test tube was drawn out to a capillary (2 mm. external diameter). The assembly was clamped so that the tip of its capillary was a few mm. above the upper end of the electrode capillary (Fig. 2*d*).

Testing the electrode

The leads from the calomel and Ag-AgCl electrodes were connected to a Cambridge pH meter (Cambridge Instrument Company), and the latter was adjusted after rinsing the glass electrode with standard phthalate buffer (pH 4.00) and

filling with the same buffer. The pH's of a range of buffers were then determined and compared with the results obtained using the glass electrode assembly supplied by the makers of the meter. The capillary electrode was considered to be satisfactory if the results agreed within 0.02 pH units within the range 2–9. On introducing 0.3 ml. liquid at the top and closing the stopcock as soon as the liquid reached the lower end of the capillary, ample liquid remained above the capillary to ensure a junction with the agar bridge. If the tip of this bridge is < 2 mm. diameter it is impossible for it to reach and possibly to damage the narrow part of the electrode capillary.

SUMMARY

The making of a simple and robust glass electrode for pH measurements on 0.3 ml. liquid is described.

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Changes in the Protein Composition of Chick Muscle During Development

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(Received 16 February 1952)

Although the earliest systematic investigations of the proteins of muscular tissue date from the work of Fürth (1895) we still know very little concerning the composition of muscles other than those of the normal adult animal. At the present time there exists a formidable array of facts and theories relating to the composition, detailed structure and functioning of adult skeletal muscle and yet, apart from a few isolated papers in the literature, little biochemical work has been carried out upon the developing tissue. Thus, the information which is available is due largely to the work of the embryologist, cytologist and histologist and is therefore mainly descriptive in character.

The changes occurring in the appearance of the muscle cell from the myoblast to the adult myofibril are reasonably well known. In the chick, with which this study is concerned, fibres can be observed in the premuscular mass of the limb buds on the 6th day of incubation which, by the 8th day, have become striated and contractile (Hunt, 1932). In the adult,

the muscle fibres are composed of numerous myofibrils of diameter about 1 μ . embedded in a viscous medium, the sarcoplasm. Connective tissue fibres of collagen and elastin ramify throughout the muscle. The cell nuclei lie under the sarcolemma, having moved there during development, from a central position in the fibre.

The intracellular proteins of the adult tissue may be conveniently divided into the proteins of the sarcoplasm, such as myogen, myoalbumin, and globulin X, which together contain all the enzymes of the glycolytic cycle; and the proteins of the myofibril, such as actin and myosin. The former are extracted from minced muscle by dilute salt solutions at a neutral pH and are characterized by their distinctive physicochemical properties. The myofibrillar protein, actomyosin, is extracted only by strong salt solutions and precipitates on dilution. If extraction is prolonged at an alkaline pH then the actin content of the extract rises and precipitation on dilution yields an actin-rich actomyosin complex.